A LEAF SPOT DISEASE OF PHYSOSTEGIA

by

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INTRODUCTION

In the fall of 1950, a leaf spot disease of Physostegia virginiana Benth. was observed at Manhattan, Kansas. Diseased plants were found in several gardens throughout the town and in the formal gardens east of Dickens Hall, on the campus of Kansas State College. This leaf spot disease, caused by Septoria physostegiae Ell. and Everh., is very damaging and can destroy an entire planting of the host. Up to the present time, very little has been known about the disease and it has continued to exist unchecked wherever an infestation has occurred. It has been the purpose of this study, therefore, to try to shed some light on the nature of the disease and the causal organism, and perhaps then it will be possible to perfect a control measure.

THE HOST

The host, Physostegia virginiana, may be familiar to some by the common names of False Dragon's head, Summer Snow, Obedient Plant and Pink Spire. It is a member of the mint family and is very useful as a perennial ornamental. Perhaps it is not grown as much today as it was in the "old-fashioned" type of perennial garden, but it can be found in several plantings in the formal gardens at Kansas State College. Its chief usefullness lies in the fact that it provides an abundant array of blooms at midsummer, when most of the other ornamental species have either finished blooming or have not yet begun to blossom.

Gates (5), lists Physostegia virginiana as native to the eastern third of Kansas. According to Rydberg (14), who accepts Dracocephalum as the correct name for Physostegia, the plant is found from Quebec to Florida and Texas to Kansas. He calls it an east temperate species.

Septoria physostegiae has been reported as occurring only on Physostegia virginiana. However, the writer has found it on a specimen of Physostegia formosier Lunell. It is not known to occur on any other species and attempts to inoculate several closely related species, which will be reported in another section of this paper, were negative.

GEOGRAPHICAL DISTRIBUTION

The earliest collection of Septoria physostegiae was made by Seymour in 1881, at Channel Lake in Illinois. However, the type is based on the collection made by Davis in 1889, at Racine, Wisconsin. Subsequent collections of this leaf spotting disease have come from other locations in Wisconsin, Illinois, Kansas and New York. The New York collection on stems of Physostegia virginiana was labelled Rhabdospora physostegiae.

SYMPTOMS

Symptoms have been observed as early as the last week in May. Typically, the first symptoms appear on the lowest leaves and then progress slowly up the plant from leaf to leaf. During the growing season, symptoms appear only on the leaves.

EXPLANATION OF PLATE I

A plant of Physostegia virginiana showing young lesions on the bottom leaves, 20 days after inoculation with a spore suspension of Septoria physostegiae.

PLATE I

Infection of the leaves results in the appearance of small areas of light brown necrotic tissue, as shown in Plate I. The necrotic spots slowly enlarge and two or more may coalesce. The spots are sunken in the center and have sharply defined margins. A light chlorotic area extends about 1 or 2 millimeters out from the margin of the spot. As the central part of the spot dries out, it becomes lighter in color, often almost white in the center, surrounded by a brownish area which marks the limit of the spot. The necrotic areas generally extend to the leaf margin and are from 1 to 5 millimeters wide and up to 20 millimeters long, with the long axis of the spot parallel to the long axis of the leaf. Severe infections cause defoliation of the plant, as was observed in Manhattan during the summer of 1950.

About a month after the spots appear, very small carbonaceous pycnidia appear in the lesions. The pycnidia can readily be seen with the aid of a 10X hand lens. The pycnidia assume no regular arrangement on the spot and in the early stages are predominantly on the upper surface of the leaf. However, the spot can be seen on both the upper and lower surfaces of the leaf. In the late stages of infection, some pycnidia can be found in the spots on the lower surface of the leaf. Typical symptoms are shown in Plate II.

EXPLANATION OF PLATE II

Leaves of *Physostegia virginiana* with lesions caused by *Septoria physostegiae* as they appear in the fall. The leaf second from left shows the lesions as they appear on the underside.

PLATE II

CAUSAL ORGANISM

Taxonomy

This disease was first described by Ellis and Everhart (4) on leaves of Physostegia virginiana, from a collection made by Dr. J. J. Davis. Their work consisted of a short description of the symptoms and the causal organism which they called Septoria physostegiae. Since their original description was published in 1889, no work, to the author's knowledge, has been done on the causal organism or the suscept-pathogen relationships. These problems have been the basis of this study.

The preliminary identification of the causal organism was made from spores found in pycnidia which were removed from lesions on infected leaves. These pycnidia were found to contain typical Septoria type spores. The pycnidia are subepidermal, erumpent, dark brown or black in color, with a thick, hard wall. The wall of the pycnidia is pseudoparenchymatous and two to three cells thick; at first light brown in color, later becoming dark brown or black and thick walled. The pycnidia have a globose-flask shape and range in size from 92 microns to 181 microns high and 111 microns to 190 microns wide. There is a short but definite beak present. The pycnophores arise from a layer of globose, thin-walled cells which line the interior of the pycnidia.

The pycnospores are filliform, straight or very slightly curved, tapering and rounded at both ends. They are hyaline and from 1 to 5 septate, depending on the maturity of the spore.

Measurements of the spores showed them to range from 18.5 microns to 33.0 microns long and 1.0 micron to 1.5 microns wide. Under favorable conditions of humidity, they emerge from the pycnidium joined in a mass to form a spore tendril.

In a moist chamber, spores ooze from overwintered pycnidia after 24 hours. A longer time was necessary before spores would ooze from pycnidia on infected leaves collected in the fall. Young lesions, in the spring, which had not developed pycnidia, produced pycnidia containing mature spores after 4 days in a moist chamber.

The pycnospores are borne singly from the small end of Indian-club shaped pycnophores which line the interior of the pycnidium.

The type and paratype specimens of Septoria physostegiae from the Ellis herbarium, New York Botanical Gardens, and two collections of the fungus from the United States Department of Agriculture herbarium at Beltsville, Maryland, were examined. The symptoms and the measurements of the critical features of the causal organism from each collection agreed with those of the specimens collected here at Manhattan.

Examinations of the type and paratype collections and an 1881 collection from the United States Department of Agriculture herbarium, showed the presence of a perithecial stage of a fungus closely associated with the pycnidial stage of Septoria physostegiae. These perithecia were excised from the same lesions that bore the pycnidia of Septoria physostegiae. The perithecia are dark brown to black, globose-flask shaped, erumpent, and aver-

age 69 microns by 78 microns in size. The asci average 38 microns long and 8 microns wide, have a slightly thickened apical portion and an apical pore. The ascospores are hyaline, two-celled, elliptical and range from 10.0 to 11.0 microns long by 2.3 to 3.0 microns wide. Paraphyses are absent. The morphology of this organism should put it in the genus Mycosphaerella, but no definite determination has been made.

The organism was also found closely associated, as above, with Septoria physostegiae on leaf lesions of a herbarium specimen of Physostegia formosier. This frequent close association gives rise to a strong suspicion that this organism is the perfect stage of Septoria physostegiae, but since a living specimen of it is lacking, this suspicion cannot be substantiated.

In 1945, Jenkins (7), reporting on a Cercospora leaf spot of Physostegia, announed that the perfect stage of that organism is a species of Mycosphaerella. The description of this Mycosphaerella agreed very closely with the perithecial stage of the fungus which was found by this writer associated with the Septoria physostegiae. However, the conidial stage of the Mycosphaerella physostegiae described by Jenkins (7) could not be found on the leaves which were examined. The conidial stage that he worked with was a species of Cercospora and should have been plainly evident had it been present on the leaves which were examined. Therefore, it is not believed that this organism found by the writer is the same as the Mycosphaerella that Jenkins described in his paper.

In an excicatti set, Fungi Columbiani, distributed by Bartholomew in 1910, a specimen (number 3379)labelled Rhabdospora physostegiae Peck n. sp. was included. This is an invalid name, since it was neither published nor was a description given of the organism, which the Botanical Rules of Nomenclature require.

Furthermore, there is evidence which indicates that the organism that Peck called Rhabdospora physostegiae on dead stems of Physostegia, is actually Septoria physostegiae. A detailed examination of the pycnidia and the spores showed them to be morphologically identical with Septoria physostegiae. Similar material collected on overwintered stems have produced leaf lesions identical to those caused by spores removed from pycnidia which were formed on leaves. Pycnidia as they appear on an overwintered dead stem are shown in Plate III.

Apparently, Peck was employing the system of classification which separates Rhabdospora from Septoria on the basis of position on the host. However, there are those who are opposed to this system and in an article (5) Garman and Stevens discuss this problem and conclude that, "The property of forming spots on leaves, or of growing parasitically upon the stems of plants is not a really valid character for the separation of genera."

Since both organisms are morphologically identical, and spores formed on stems will cause infection on leaves, and vice versa; and, furthermore, since the name was never validly published, the nomenclature and synonymy are as follows:

EXPLANATION OF PLATE III

A portion of an overwintered stem (much enlarged) showing pycnidia that developed during the winter.

PLATE III

Septoria physostegiae Ellis and Everhart
Journal of Mycology 5: 150. 1889.
Rhabdospora physostegiae Peck (nom. nud.)
Bartholomew. Fungi Columbiani (number 3379) 1910

MATERIALS AND METHODS

The causal organism was at first rather difficult to isolate in pure culture. Work was begun with the organism in September of 1950, and due to the fact that the pycnidia are not all fully mature until the spring, the spores would not ooze out for several days after leaves were placed in a moist chamber. However, the secondary invaders which were present grew and sporulated profusely, thus contaminating the area and making isolation difficult. A further complicating factor was that the pathogen that was being isolated grows rather slowly and was inhibited by most of the common contaminants. Repeated attempts to isolate from necrotic tissue or spore tendrils failed because of the above reason.

The pathogen was finally isolated by using pycnidia which were excised from the host tissue, thereby eliminating most contaminants. The small size of the pycnidia made them very difficult to work with, and it was, therefore, necessary to sterilize them on the leaves. The process evolved consisted of first washing the leaf with a stream of tap water, to physically remove surface dirt; then the leaf was rinsed in 95 Percent alcohol; and next it was immersed in a 1/1000 mercury bichloride solution for 30 seconds. After the mercury bichloride sterilization, the

leaf was thoroughly rinsed with sterile water, and then, using a binocular microscope and a sterile needle, several pycnidia were excised, with care taken to get them as free of host tissue as possible. Generally the larger pycnidia came free of the host tissue quite easily. The excised pycnidia were put into a drop of sterile water on a sterile glass slide and then crushed with a sterile needle, thus liberating the contained spores. The spore suspension was then plated out with a transfer loop to petri dishes of potato dextrose agar. The success of this process was due to the fact that the spores within the pycnidium were protected while all the contaminants on the outside were killed by the sterilizing agents. It was also very fortunate that potato dextrose agar was used for these primary isolations, because on this media, the growth of the organism was so different and distinct that it was immediately recognized as not one of the common contaminants which had been previously encountered.

In the preliminary phases of this work, potato dextrose agar was used, but later findings caused a change to corn meal agar for all stock cultures and routine work. Several other types of media were used in the experimental work and a short discussion of them is given below.

The following media were made up according to formulae in Riker's Manual (13): Potato dextrose agar, corn meal agar, oat agar, malt agar and water agar. Physostegia agar and Coleus agar were also made up according to a suggested formula in Riker's Manual. Fresh plant material was ground up in a Waring blendor and

then added to water in which agar had been dissolved. The media was then autoclaved. The Coleus and Physostegia agars contained 15 Percent plant material and 20 grams of agar for every liter of distilled water.

A lima bean agar was made from a Difco prepared product.

Czapek's solution agar was made up according to a formula given by Raper and Thom (11).

A medium developed by Gorodkowa (15) which stimulates yeast sporulation was also used, and contains the following ingredients:

Glucose	2.5 grams
Sodium chloride	5.0 grams
Meat extract	10.0 grams
Agar	10.0 grams
Water	1.0 liter

The hydrogen-ion concentration of the media which were used was checked and found to be as follows:

Potato dextrose agar	5.5
Corn meal agar	5.65
Lima bean agar	5.4
Malt agar	5.15
Coleus agar	5.55
Physostegia agar	5.5
Water agar	6.5
Czapek's medium	6.85

The hydrogen-ion concentration of media used in studying the relation of acidity and alkalinity to growth in culture was adjusted

by the addition of acetic acid or potassium hydroxide.

Since most of this work was conducted during the winter months, the plants which were used in the experimental work were grown in pots in the greenhouse. These plants were grown from seeds and from rhizomes purchased from a commercial seedsman.

FUNGAL ASSOCIATIONS

During the course of these investigations, under the routine circumstance of isolating and culturing Septoria physostegiae from diseased field material, the presence of other fungus species was noted. One in particular, Alternaria tenuis auct. sensu Wiltshire, occurred with almost constant regularity in the isolations from older lesions of leaf spots, and because of its rapid growth, made the task of getting Septoria physostegiae into culture somewhat more difficult.

The other associated fungi were a species of Phyllosticta, and a species of Stagnospora. Beyond these observations, no further information is available as to the nature of the fungal associations.

CULTURAL CHARACTERISTICS

Relation of Media to Growth in Culture

Organisms have been cultured on various media for numerous reasons by many workers. The isolates of Septoria physostegiae were grown on various culture media as an aid to identification

and as a possible means of inducing the ascigerous stage of this organism. The formulae for the media used is the same as that given in the "Materials and Methods" section of this paper.

The first attempt at this phase of the work failed because contaminants readily entered the petri dishes when they were moved about in the process of examining them. It was then decided that, after the substrate was inoculated with the organism, the dishes were to be sealed with a cellulose tape. Petri-dish cultures of all media were made in quadruplicate and held at a fluctuating room temperature for 40 days. During, and at the end of this period, observations on cultural characteristics were recorded. Plate IV shows one set of these cultures as they appeared at the end of the 40 days. Color determinations were based on Ridgway's "Color Standards and Color Nomenclature" (12). A culture growing on corn meal agar was divided into small pieces about 1 millimeter square, and these were used as the inoculum to start the cultures in this series. Eight kinds of media were used and the resulting cultural characteristics which were observed on seven are described below. The measurements in millimeters which are given refer to the average diameter of the colony on the particular medium at the end of 40 days.

Potato Dextrose Agar. Growth on potato dextrose agar was the most unusual of all. The organism formed a pulvinate colony which was much wrinkled and covered with a very close, dense layer of fine short hyphae. The presence of large pseudopycnidia gave a warty appearance to the surface. The organism never produced

pycnospores growing on potato dextrose agar. Colonies grew to an average size of 17 millimeters. The border of the colony was deep neutral gray and the aerial mycelium toward the center, light seal brown, while the mycelium forming the stromatic mass was fuscous-black.

Corn Meal Agar. On this medium, the organism formed a slightly raised stromatic colony, which was made up of closely packed mycelium. Very fine gray (Pale Gull Gray) aerial mycelium formed a tufted mat over the stromatic mass of dark neutral gray surface mycelium. Colonies grew to an average of 36 millimeters and produced pycnidia imbedded in the stromatic mat, which matured spores 20 days after the colonies were started.

Malt Agar. Growth on this medium was similar to that on potato dextrose agar. However, the stromatic mass was not raised as high as it was on potato dextrose agar. The olivacious black mycelium toward the center of the colony was dense and long, giving the colony a hedge-hog appearance. Aerial mycelium around the edge of the colony was iron gray. Mature spores were found in pycnidia 25 days after the colonies were started. An average of 28 millimeters growth was made.

Lima Bean Agar. The organism on this medium showed a tendency to produce sectors of the colony which varied in color and amount of aerial mycelium. The normal colony was a flat stromatic mass of closely packed dusky neutral gray mycelium with a very short overgrowth of aerial mycelium. Pycnidia were produced which bore mature spores about 40 days after the colonies were started. Colonies averaged 21 millimeters in diameter.

Coleus Agar. On this medium, the organism made a flat, spreading type of growth. No true stromatic mat was formed. Surface mycelium was dark, neutral gray and the cottony tufts of aerial mycelium which were scattered over the surface were white. Numerous pycnidia bearing mature conidia were found about 25 days after the colonies were started. The pycnidia were black, borne on the surface and clearly evident. Colonies averaged 25 millimeters.

Physostegia Agar. Septoria physostegiae made about the same type of growth on this medium as it did on the one above. However, the surface mycelium, which was also dark neutral gray, grew more densely and produced a slight stromatic type of mat. Aerial mycelium was abundant and appeared to be light olive gray in color. Spores were produced on this medium 30 days after the colonies were started. An average of 21 millimeters of growth was recorded.

Czapek's Medium. The same general type of growth was made on this medium as was observed on most of the other media; a flat stromatic colony formed by a mat of closely growing mycelium. However, the black color of the mat, lack of aerial mycelium and limited amount of growth (only 6 millimeters) made the growth on Czapek's medium very distinctive. No pycnidia were formed. The colony radiated out in finger-like lobes, rather than in concentric rings.

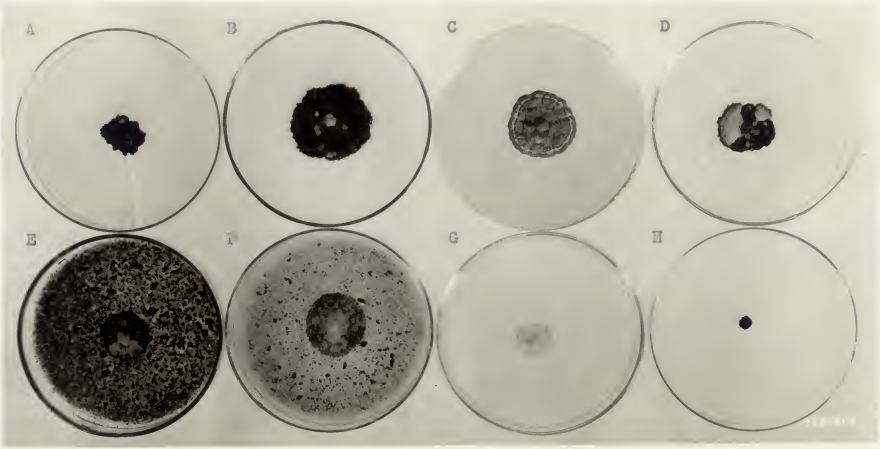
Water Agar. The only growth made on this medium was a sparse, arachnoid type of mycelium. No stromatic mat was formed, and the

EXPLANATION OF PLATE IV

The organism, Septoria physostegiae, as it appears growing on different agar media.

A. Potato dextrose agar
B. Corn meal agar
C. Malt agar
D. Lima bean agar
E. Physostegia agar
F. Coleus agar
G. Water agar
H. Czapek's medium

PLATE IV



growth did not range much beyond the original piece of inoculum. The mycelium was white and spread, at the most, to 10 millimeters. No pycnidia were formed.

Gorodkowa's medium was also used but growth was so limited on this medium that no record of it was made.

Relation of Temperature to Growth in Culture

Cultures were set up in the same manner as described in the previous section. Three petri dishes of each media were then kept at the following temperatures: 5° C., 25° C., 37° C. and room temperature. The cultures were kept at these temperatures for 3 weeks and then examined.

Observations indicated that temperatures had no effect on the type of growth made by the organism on the different media. However, temperature did affect the amount of growth that was made, which was measured by the amount of increase in diameter of the colony. All cultures were affected in a like manner by the various temperatures.

The greatest amount of growth was made at both 25° C. and room temperature. The organism grew very slowly at 5° C. and at 37° C. there was practically no growth at all. It is possible that the drying effect of the higher temperature caused the reduced amount of growth.

Relation of Acidity and Alkalinity to Growth in Culture

Several series of petri dishes containing corn meal agar

were prepared, their hydrogen-ion readings ranging from pH 4.5 to pH 8.0. Pieces, about 1 millimeter square, of a colony grown on corn meal agar, were transferred to these dishes and incubated at 25° C. for 23 days.

It was found that there was no difference in the amount or type of growth that was made at each of the different hydrogen-ion concentrations. However, there was a difference in the production of pycnospores. Microscopic examination revealed that no spores were produced below pH 5.0 or above pH 7.0, but at the hydrogen-ion concentration of 5.0 to 7.0, abundant pycnospores were found.

Cultural Variants

On numerous occasions, when cultures of Septoria physostegiae were grown on lima bean agar, a white variant, showing considerable aerial mycelium, made its appearance. In these cultures, the variant is pallid, neutral gray, while the rest of the colony is iron gray. This type of variant, appearing as a sector, can be seen in Plate IV, D.

The variant was subcultured by mycelial transfer and continued to show the different habit of growth. A parallel culture made from the dark portion of the colony averaged 5 centimeters of growth, while the variant averaged 9 centimeters in the same period of time. Also, the variant produced spores after 12 days of growth, while the normal colony did not.

Evidence for mutation in fungi, in the sense of genic alter-

ation, is not readily adduced in the absence of a sexual stage. And yet, the white form of the fungus, as described above, came into view as a variant in a pure culture of Septoria physostegiae, and has made spontaneous appearance on several separate occasions. Furthermore, it can be maintained in pure culture and shows no further indication of instability. It, then, is regarded as a mutant of Septoria physostegiae.

PATHOGENICITY

The fact that an organism was found to be present on leaves of a plant does not indicate pathogenicity by that organism. Therefore, in order to prove the pathogenicity of Septoria physostegiae for Physostegia virginiana, inoculations were made and Koch's rules of proof were followed.

Spores from pure cultures, growing on corn meal agar, which had been isolated from pycnidia removed from leaf lesions, were used as the inoculum. Inoculations of the leaves were made in two ways. In one, the spores were suspended in sterile water, and with an atomizer, sprayed on the leaves of the plant. The other method of inoculation consisted of painting a drop of spore suspension on the leaf with a platinum loop. The plants were covered with bell jars for 7 days immediately following inoculation and then uncovered and allowed to remain on the greenhouse bench. Fourteen days after inoculation, minute light brown necrotic areas began to appear on the leaves. These spots developed into typical leaf spot lesions. Isolations from these necrotic areas produced

the fungus in culture and these cultures agreed in all respects with the original material. Also, after 40 days, mature pycnidia developed in the necrotic areas. Pycnospores, which compared in every respect with the pycnospores originally used, were isolated from the pycnidia on the artifically produced lesions. They were cultured with a parallel series of daughter cultures from the original culture which was used for inoculum, and no differences were noted.

Inoculation experiments were conducted with five other members of the mint family. Four of these, Dracocephalum nutans L., Dracocephalum ruyschiana L., Phlomis fruticosa L. and Nepeta Mussinii Spreng. are listed as closely related to Physostegia virginiana by Bailey (1). The fifth plant used was Coleus blumei Benth. None of these plants became infected by Septoria physostegiae.

SPORE GERMINATION

To observe the manner of spore germination, Van Teighm cells were used. A drop of filtered corn meal agar was placed on sterile cover slips and allowed to harden for several hours. Then, with the aid of a transfer loop, the drops of hardened agar were inoculated with a drop of sterile water containing an abundance of spores. The cover slips were then inverted and sealed to the depression slides with petroleum jelly.

Four slides were held at each of the following temperatures: 5° C., 25° C., 37° C. and room temperature. Each slide at each temperature was observed at various time intervals and the slide

at room temperature was placed in focus on a microscope and kept there for 4 days, so that camera lucida drawings could be made to record, at intervals, the method and process of germination. Plates V and VI show camera lucida drawings which trace the developmental steps of a germinating spore.

Observations after 12 hours of incubation showed that the spores held at 25° C. and room temperature had swollen to approximately 3 times their original size, while those at 5° C. and 37° C. had not changed at all. At the end of 16 hours, the spores at 25° C. and room temperature began to germinate. Germ tubes emerged from cells at either or both ends of the conidia and often from other cells as well. Early growth of the hyphae was rapid, much elongated and with profuse branching. Plate VII shows camera lucida drawings of resting spores and spores after 12, 14 and 16 hours of incubation.

The spores held at 5° C. failed to germinate but were swollen to twice their original size after 4 days and remained that way as long as they were kept at 5° C. One slide of spores that had been held at 5° C. for 4 days was removed to room temperature and germinated after 11 hours.

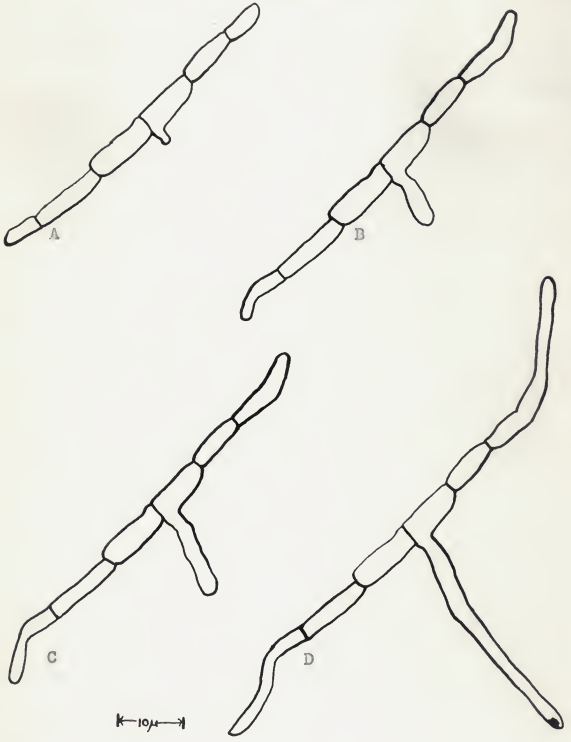
The spores which were held at 37° C. failed to germinate and showed no swelling at all. Even after removal to room temperature, (after being held for 4 days at 37° C.) they failed to germinate.

EXPLANATION OF PLATE V

Camera lucida drawings of a spore germinating on corn meal agar, at room temperature.

A. 14 hours after incubation
B. 16 hours after incubation
C. 18 hours after incubation
D. 20 hours after incubation

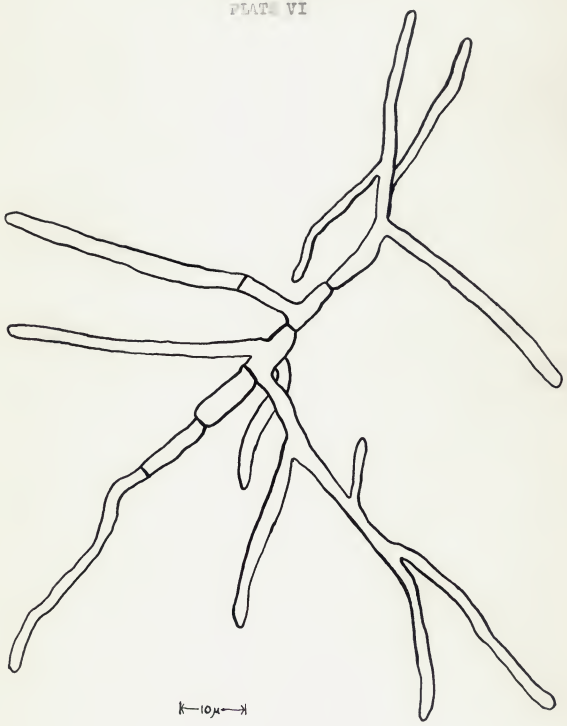
PLATE V



EXPLANATION OF PLATE VI

Camera lucida drawings of same spore shown in Plate V, 25 hours after incubation.

PLATE VI



EXPLANATION OF PLATE VII

Camera lucida drawings of spores of Septoria physostegiae:

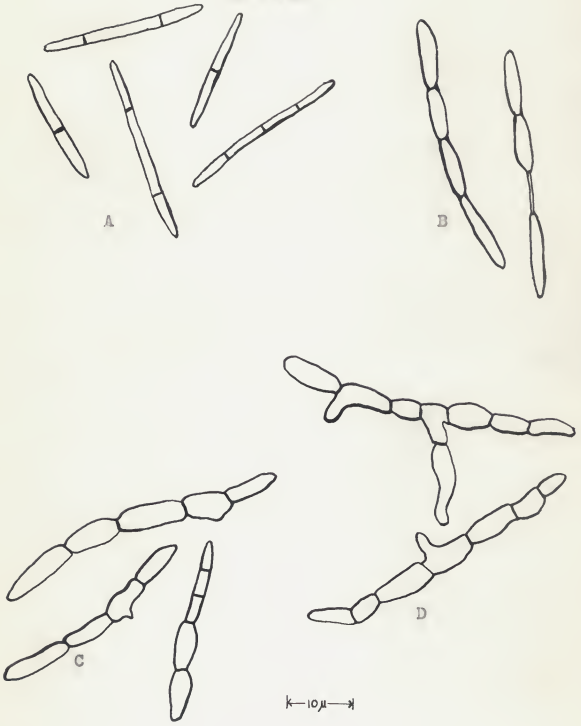
A. Spores in the resting stage, showing the variation in size and septation.

B. Spores, 12 hours after incubation, having swollen to 3 times their original width. One spore shows a cell which has not swollen.

C. Spores, 14 hours after incubation, having begun to show buds which will develop into lateral branches. One spore shows 3 cells which have not completed swelling.

D. Spores, 16 hours after incubation, lateral branches having begun to develop.

PLATE VII



MODE OF INFECTION

The mode of infection was studied on leaves of Physostegia plants grown in the greenhouse. The staining and fixing technique employed is a modification of methods used by both Bollard (3) and Paddock (10).

Areas about 2 millimeters square were marked with four small dots of India ink on both the upper and lower leaf surfaces. A drop of a water suspension of pycnospores was placed on each square by means of a platinum loop. The plants were placed under bell jars immediately after inoculation and left there until the final leaf samples were taken. One of the marked squares from both the upper and lower leaf surfaces was removed and placed in a warm solution of equal parts of absolute alcohol and glacial acetic acid at the following time intervals after inoculation: 19, 32, 41, 55, 65, 81, 103, 127 and 137 hours. They were left in this solution for at least 24 hours and then submerged for 5 minutes in lactophenol. Then they were immersed in a saturated lactophenol-cotton blue solution for 2 minutes. The excess material was washed from the tissue with clear lactophenol and it was then mounted on a slide in a drop of lactophenol and examined under the microscope. The pycnospores and mycelium were stained a dark blue but the host tissue was clear and translucent.

By examination in this way, the extent of growth and place of penetration were readily seen. At the end of 32 hours, the spores germinated on the leaves. The manner of germination is similar to the germination of spores on agar, but there are some

minor differences. The spores swell to only twice their original size before germination and the cells of the primary hyphae are more elongate. Also, the mycelium branches a great deal more on the agar than on the leaf.

Examination of a large number of pieces of leaf tissue prepared in the above manner showed that germ tubes entered the leaf tissue, both upper and lower surfaces, through the stomates. The earliest penetration was found to occur 81 hours after inoculation. In many instances, germ tubes were seen to grow right over a stomate without penetrating at that point. All cases of penetration were characterized by an appressorium-like enlargement which comes to lie immediately in the depression which delimits the outer mouth of the stomate. From this enlargement, a further development occurred; a tube, much reduced in diameter formed, which continued to penetrate the leaf tissue. No penetrations through other than open stomates have been observed.

PATHOLOGICAL HISTOLOGY

The pathological histology was determined from infected leaves of Physostegia virginiana which were removed from plants grown in the greenhouse. Material was chosen which contained necrotic, partially necrotic and healthy tissue. The material was prepared for study following methods given by Johansen (8). It was killed and fixed in a formalin-aceto-alcohol fluid, next dehydrated by a tertiary butyl alcohol method and then infiltrated with parowax. The following combinations of stains were

tried: Safranin and fast green; Thionin, light green, and orange G plus erythrosin; Methyl violet and eosin.

It was found that the safranin and fast green combination was the easiest to use, and gave the clearest and best results. With this stain, the healthy host tissue was a light green, the fungus a medium red, and the necrotic tissue a dark red color.

In the necrotic areas, the tissue was disorganized, shrunken and collapsed. The cell walls appeared thicker than in the adjacent healthy tissue. The mycelium in the necrotic areas was both inter and intracellular.

Mycelium of the causal organism was observed to extend from 1 to 2 millimeters beyond the necrotic tissue and into the healthy tissue. In the healthy tissue, the mycelium was found to be only intracellular.

PERFECT STAGE

One of the primary objectives of this work was to find or produce the ascigerous stage of the causal organism, Septoria physostegiae. The several methods which were used will be described below; however, all failed to produce the perfect stage.

Infected leaves were put into a wire basket which was placed on the ground in a garden. It was left there all winter, and finally, in April and at intervals thereafter, some of the material was examined. The only change that could be found was that whereas in the fall, the pycnidia were confined to the necrotic lesions, now they could be found distributed all over

the upper and lower leaf surfaces and on the stems.

Stem and leaf tissue of Physostegia virginiana was partially imbedded in water agar in test tubes, and then sterilized. The tissue was then inoculated with Septoria physostegiae, and allowed to remain at room temperature for 5 days. Three tubes were not inoculated and were kept as checks. The tubes were kept at room temperature for 5 days, to allow the fungus to become established in the host tissue. After the 5-day period, ten of the inoculated tubes and one check were placed in a refrigerator maintained at 5° C., and two of the inoculated tubes and one check were kept at room temperature.

Every other week, one of the tubes which had been kept at 5° C. was removed and examined and then kept at room temperature. At the end of 20 weeks, none of the tubes had produced an ascigerous stage, but all had produced mature pycnidia.

Septoria physostegiae was also cultured on Coleus stems and leaves in a manner similar to that described above. However, these cultures were all maintained at room temperature. The organism grew on this material but produced few mature pycnospores.

The organism was also grown on various media at four different temperatures. The media used were potato dextrose agar, corn meal agar, oat agar, malt agar, lima bean agar, Coleus agar, Physostegia agar, water agar, Czapek's agar and Gorodkowa's medium. Four replicates of each medium were set up and one of each was kept at 5° C., 25° C., 37° C. and room temperature.

It was thought the pH might be the controlling factor, so a

series of cultures using corn meal agar and ranging from pH 4.5 to 8.0 were prepared. The results from this attempt, like the others, were negative.

Acting on a suggestion by Professor L. E. Melchers, test tube slants of corn meal agar which were inoculated with Septoria physostegiae were placed on a north-facing window sill. The cotton plugs were protected from rain by a piece of paper attached over the wire basket containing the tubes. It has been the experience of Professor Melchers, and others, that these conditions have stimulated other organisms to produce the perfect stage. However, the only effect noticed in this instance was the rapid and abundant production of the conidial stage. In fact, only 5 days after the cultures were started, spores were seen oozing from pycnidia. It is possible that these conditions will produce the perfect stage at another time of the year, with different temperature conditions.

CONTROL

No control measures were attempted. However, since pycnospores which overwinter in pycnidia are probably the primary inoculum, the removal of all material bearing the pycnidia would reduce the source of inoculum and thereby reduce the incidence of this disease. In the fall of the year, care should be taken to remove and burn not only all old Physostegia leaves, but also all old stems. Stubble, only 2 or 3 inches long, can serve as the source of inoculum for next year's infections. Therefore,

sanitation must be thorough to be effective.

SUMMARY

A leaf spot disease of Physostegia virginiana, caused by Septoria physostegiae, was found to be causing a serious defoliation of Physostegia plants during the summer of 1950. The disease occurs only on Physostegia and has been reported from Michigan, Illinois, New York, and now Kansas.

The symptoms, necrotic spots, occur only on the leaves, and are readily identified by the small, carbonaceous pycnidia which develop in them. Measurements of the causal organism show the pycnidia to average 137 microns high and 150 microns wide, and the pycnospores, which are hyaline and 1 to 5 septate, average 25 microns long and 1.2 microns wide. The ascigerous stage is unknown, but a frequently encountered species of Mycosphaerella may prove to be it. An organism, called Rhabdospora physostegiae has proven to be Septoria physostegiae and has been put in synonymy with it.

A constant and bothersome fungal associate was a species of Alternaria, which has been called Alternaria tenuis.

The cultural characteristics were found to vary with each of eight different media used. Corn meal agar was considered best for culturing the fungus and the optimum temperature for growth in culture was either 25° C. or room temperature. Hydrogen-ion concentrations between 4.5 and 8.0 did not alter the cultural characteristics of the organism, but the spore production was inhibited below 5.0 and above 7.0.

Sectoring, which indicated a cultural variant, occurred frequently on lima bean agar. Although the sexual cycle is needed for genetic proof, it is felt that the variant is a mutant of Septoria physostegiae.

At the optimum temperatures of 25° C. and room temperature, spores germinated in 16 hours on corn meal agar. However, it took 32 hours for the spores to germinate on leaves. Germ tubes penetrated leaves through stomates 81 hours after inoculation. Appressorium-like enlargements were seen to occur where the germ tubes entered the stomates. Necrotic spots began to appear 14 days after inoculation. Mycelium of the causal organism extended beyond the necrotic tissue and grew intracellularly, except in the necrotic tissue, where it was both intra and intercellular.

Pycnospore production was most rapid on corn meal agar in tubes kept on a north exposure window sill.

No control measure was tested, but it is thought that sanitation in the fall would be all that is necessary.

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A LEAF SPOT DISEASE OF PHYSOSTEGIA

by

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ABSTRACT OF THESIS

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Serious leaf spotting, and subsequent defoliation of a perennial ornamental, Physostegia virginiana Benth., has been caused by the fungus Septoria physostegiae Ell. and Everh. The host plant, most commonly known as False Dragon's Head, is a member of the mint family and is native to the eastern third of Kansas.

This disease occurs only on Physostegia and has been reported from Michigan, Illinois, New York and now Kansas. The first reported collection was made by Seymour in 1881 at Channel Lake in Illinois, but the type specimen used by Ellis and Everhart for their description of the organism was based on a collection made by Davis in 1889, at Racine, Wisconsin.

The symptoms, necrotic spots, occurred only on the leaves, mostly the upper surface, and appeared as light brown areas with sharply defined margins. Late in the summer, small carbonaceous pycnidia developed in the spots. The pycnidia averaged 137 microns high and 150 microns wide. The pycnophores, which line the interior of the pycnidia, are Indian-club shaped. Pycnospores, which were born from the small end of the pycnophores, were hyaline, 1 to 5 septate and averaged 25 microns long and 1.2 microns wide. The above measurements of the critical features agreed in every detail with measurements of these same features of the type specimen.

The ascigerous stage is unknown. However, several of the herbarium specimens that were studied showed a species of Mycosphaerella occurring on the same lesions with Septoria physostegiae.

It is strongly suspected that this is the perfect stage of the causal organism.

The species Rhabdospora physostegiae has proven to be Septoria physostegiae and has been put into synonymy with it.

Several fungal associates were observed to occur with Septoria physostegiae. Alternaria tenuis auct. sensu Wiltshire, because of its almost constant occurrence, made the task of isolating the causal organism very difficult.

The causal organism was grown on the following media: Potato dextrose agar, corn meal agar, malt agar, Coleus agar, Physostegia agar, water agar, Czapek's medium, lima bean agar and Gorodkova's medium. Cultural characteristics were observed to vary with each media that was used. Corn meal agar was considered best for culturing the fungus, and either 25° C. or room temperature gave optimum growth.

Several methods for producing the perfect stage were attempted, none of which succeeded. However, during the course of this work, it was learned that hydrogen-ion concentrations between 4.5 and 8.0 did not alter the cultural characteristics of the organism, but spore production was inhibited below pH 5.0 and above pH 7.0. Also, pycnospore production was most rapid on corn meal agar slants kept on a north exposure window sill.

Sectoring, which indicated a cultural variant, occurred frequently on lima bean agar. Although the sexual cycle is needed for genetic proof, it is felt that the variant is a mutant of Septoria physostegiae.

Spore germination was observed on hanging agar drops and on Physostegia leaves. At the optimum temperatures of 25° C. and room temperature, spores germinated in 16 hours on corn meal agar. However, it took 32 hours for the spores to germinate on leaves. Germ tubes penetrated leaves through stomates 81 hours after inoculation. Appressorium-like enlargements were seen to occur where the germ tubes entered the stomates. Necrotic spots began to appear 14 days after inoculation.

The pathological histology was investigated using a safranin and fast green stain combination. Mycelium of the causal organism grew intracellularly beyond the necrotic tissue. In the necrotic tissue, it was both intra and intercellular.

No control measures were tested, but sanitation in the fall is thought to be all that would be necessary.